IMPROVEMENTS IN UNDERSTANDING EXPOSURE AND TOXICITY ISSUES ASSOCIATED WITH RDX: RECENT UPDATES

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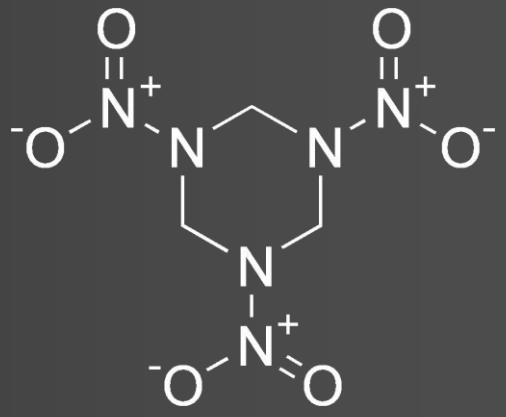
Outline

- Background
- The regulatory issues
- Recent advances
  - Cancer reassessment
  - Non-cancer reassessment
  - Mode of action in the development of neurotoxic effects
  - Physiologically-based, pharmacokinetic (PBPK) models
  - Reassessment of the relative source contribution in groundwater

Background



- RDX ,1,3,5-trinitrohexahydro-1,3,5-triazine is an explosive with widespread application as a component in propellants, detonators, grenades, bombs and a variety of other military ordnance.
- RDX has become a contaminant at military bases in the U.S. Inadvertent accumulation of low order/incomplete detonations on ranges has resulted in environmental contamination in soil with concentrations detected in groundwater.
- Issues – Oral exposures from RDX-contaminated groundwater. Log K_{ow} = 0.87, low affinity for carbon; water solubility = 60 mg/L

Current USEPA Criteria

- Possible human carcinogen - hepatocellular carcinomas in female (not male) B6C3F1 mice (Lish et al., 1984). Oral Slope Factor = 0.11 (mg/kg)/d.
- Oral Referenced Dose (RfD) of 3 μg/kg/d is based on incidence of prostatic inflammation in a 24 month rat study (Levine et al., 1983; NOAEL = 0.3 mg/kg/day).
- Longer-Term Health Advisory (LTHA) for RDX of 0.35 mg/L is based on the No-Observed-Adverse-Effect-Level (NOAEL) in seizing monkeys (Martin and Hart, 1974). Lifetime HA = 2 μg/L/d based on prostate inflammation in geriatric rats.
- Acute oral Minimum Risk Level (MRL) of 0.06 mg/kg/d is based on the 20 mg/kg/day dose of RDX-induced convulsions in animals (Angerhofer et al., 1986).
- Included in Contaminant Candidate Lists (CCL) 1,2 &3 but not selected for regulatory determination. RDX is not regulated by Safe Drinking Water Act. DWEL = 100 μg/L Lifetime HA = 2 μg/L

Regulatory criteria - summary

- Cancer – based on single study/single sex
- Non-cancer effects
  - Blood effects
  - Convulsions (neurological)
  - Prostate inflammation
- Federal and State drinking water values are variable.

Uncertainty Factors - RfD

- Animal to Human (Interspecies) - 10
- Sensitive Sub-population (Intraspecies) - 10
- Relative Source Contribution – 0.2
- Developmental Effects - 3
- Subchronic to Chronic Uncertainty – 10
- LOAEL to NOAEL - 10

Lowest Adverse Observed Effect Level (LOAEL) = 100 mg/kg/d

LOAEL to NOAEL = 10

Interspecies = 10

Intraspecies = 10

Subchronic to chronic = 10

RfD = 0.01 mg/kg/d

USEPA Re-evaluation of RDX

- The EPA encourages information which would refine estimates of the HA and RfD enabling a more predictive approach to determine UFs rather than a conservative protective approach using classic defaults.
- Recent, scientific information regarding toxicodynamics, toxicokinetics and mechanisms will provide a more accurate reassessment and determination of safe levels of exposure.
  - If these values remain low or become lower, training and testing activities will be adversely affected, adversely affecting military readiness.
  - If these values are artificially low, significant resources will be spent clean up costs associated with unnecessary remediation costs.

Cancer Reassessment

- Weak evidence for carcinogenicity
  - Negative results for:
    - Ames assays (bacterial mutagenicity)
    - Mammalian *in vitro* assays (mouse lymphoma & CHO)
    - Mammalian *in vivo* assays (mouse micronucleus).
    - Not teratogenic in rats or rabbits (Cholakis et al., 1980; Reddy et al., 2005).
  - Positive in the non-standard TA97a strain of *Salmonella tryphimurium* following s9 activation (Pan et al., 2007).
- Negative for cancer in chronic rat study (Levine et al. 1983).
- Positive for liver neoplasms found in female mice (Lish et al. 1984)
  - Reassessed by a team of pathologists to determine incidence of cancer using current criteria (Parker et al., 2006).
    - Lish did not define diagnostic criteria; several neoplasm were reclassified as non-neoplastic lesions using modern diagnostic criteria.
    - Used military grade (~89% RDX); changed dose midway through study.
    - Incidence of neoplasm for all groups within range of spontaneous neoplasms of female mice (incidence in Lish controls inordinately low) significance only in 35 mg/kg/day, i.e., only equivocal evidence of carcinogenicity.

Cancer slope factor

- Data for quantitative evaluation of cancer risk from RDX exposure are weak at best.

- The RDX cancer reevaluation is in process:

  Data are consistent with the descriptor:

  "Suggestive of Cancer"

- Quantitative risk assessments may soon have to be done using only the non-cancer RfD

  *Pending outcome of USEPA reevaluation

Non-cancer Reevaluation

- Evaluate endpoints
  - Neurotoxic – brain
  - Hemotoxic – blood
  - Immunotoxic – prostate
- Reduce uncertainty
  - Determine dose to target in animals
    - Extrapolate more accurately to humans
  - Determine if humans are as sensitive as animals
    - Understand mechanism
    - Understand physiological differences
- Reduce uncertainty factors
  - Reduce interspecies UF – 3
  - Provide data to support changing RSC to 0.5

SUBCHRONIC ORAL TOXICITY OF RDX IN RATS

(Crouse et al. 2006)

- Results
  - Maximum Tolerated Dose (MTD) was 20 mg/kg/d; loss of body weight, convulsions and death observed.
  - No blood effects seen at any dose; a result inconsistent with anemia reported by Cholakis et al., 1980 (90 d) and Levine et al., 1983 (2 yr; NOAEL = 0.3 mg/kg/day).
  - No evidence of immunosuppression.
  - No treatment-related prostate effects.
  - LOAEL based on neurological effects (convulsions) at 8 mg/kg/d; NOAEL = 1 mg/kg/day.

Neurotoxicity is consistent endpoint

- Convulsions consistently observed with increased salivation in mammals
  - Human - Barsotti and Crofti, 1949; Kucukardalr, 2003
  - Primates – Monkey (Martin and Hart 1974)
  - Rat - Burdette et al., 1988; Crouse et al., 2006
  - Pig – Bannon (in prep).
  - Northern Bobwhite – Quinn et al. 2008
  - Western fence lizard – McFarland et al. 2009

Mechanism of Seizure Unknown

Interspecies variation in LOAEL/NOAEL values

	Western fence lizard (mg/kg-d)	Northern bobwhite (mg/kg-d)	Mammals (mg/kg-d)
TNT	25/15	70/20	8/2
DNT (2,4/2,6)	25/15	15/5 40/10	1.5/0.2 7/nf
RDX	**5/2.5**	**8/3***	**8/4**
A-DNT	15/5	14/3	In progress
HMX	~5000/na	~5000/na	10/5

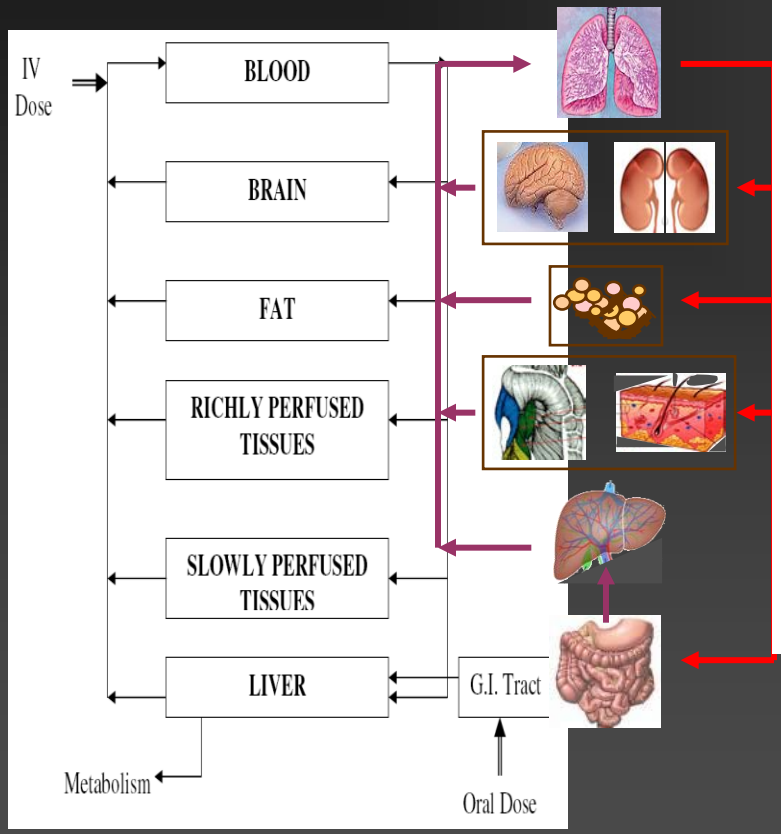
* - 14-d gavage; all others 60-d exposures.

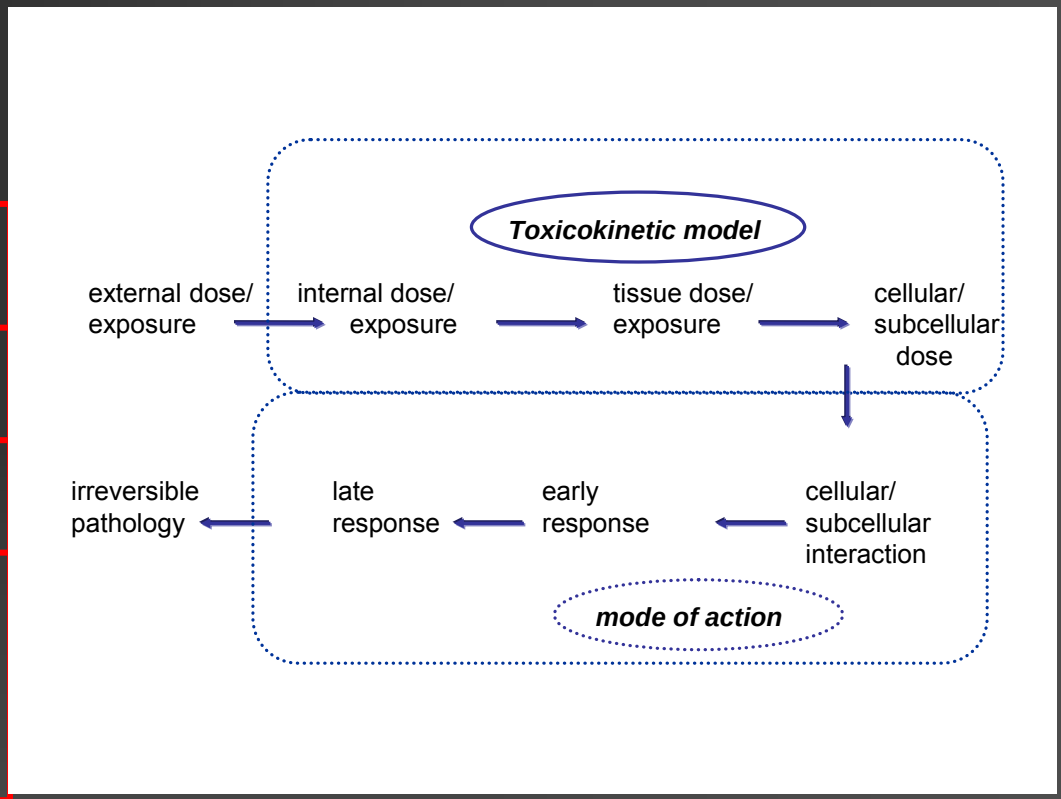
nf – NOAEL not determined

Toxicokinetics:

- Oral absorption is fast
  - Onset of seizures in rats is ~11 min at 75 mg/kg
  - Plasma concentration at seizures is unknown
  - Brain concentration in rats at seizure in unknown (~20 ppm in quail)
  - Blood/plasma coeff = 1:1
- Metabolism and excretion – Oxidative enzymes cleave ring, produce (Jackson et al., 2007; Major et al., 2007):
  - 4-nitro-2,4, diazabutanal (NDAB)
  - methylenedinitramine (MEDINA)
  - 4-nitro-2,4,-diaza-butanamide in urine

PBPK Modeling





ADME

Absorption
Distribution
Metabolism
Elimination

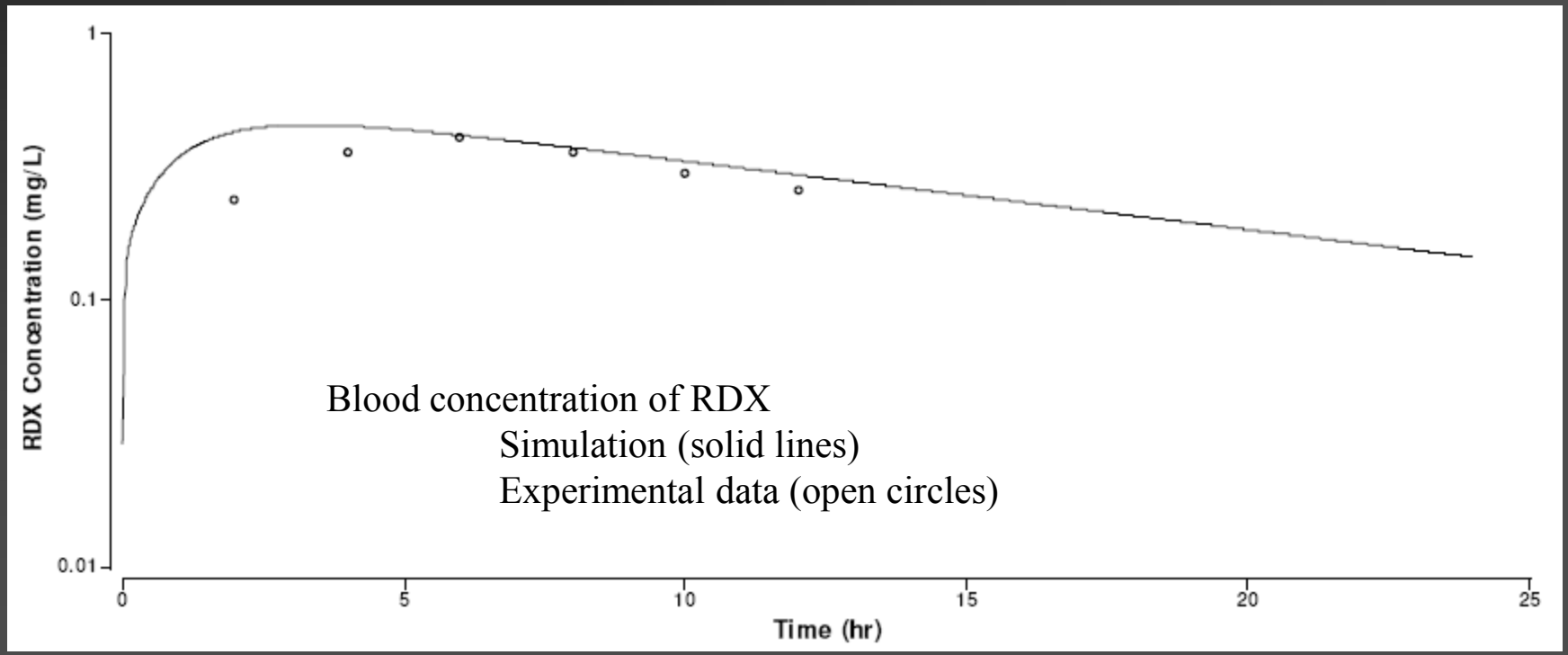
USACHPPM Readiness thru Health

Rat PBPK Model Construction – Krishnan et al., 2009

- Each tissue compartment in the RDX PBPK model was described with a mass balance differential equation (MBDE) that consisted of a series of clearance terms.
- The perfusion-limited tissue uptake of RDX in blood was described according to Fick's law of simple diffusion.
- Liver metabolism and oral absorption were described as a first order process
- Tissue:blood partition coefficients for RDX

PBPK Model Simulation - Krishnan et al., 2009

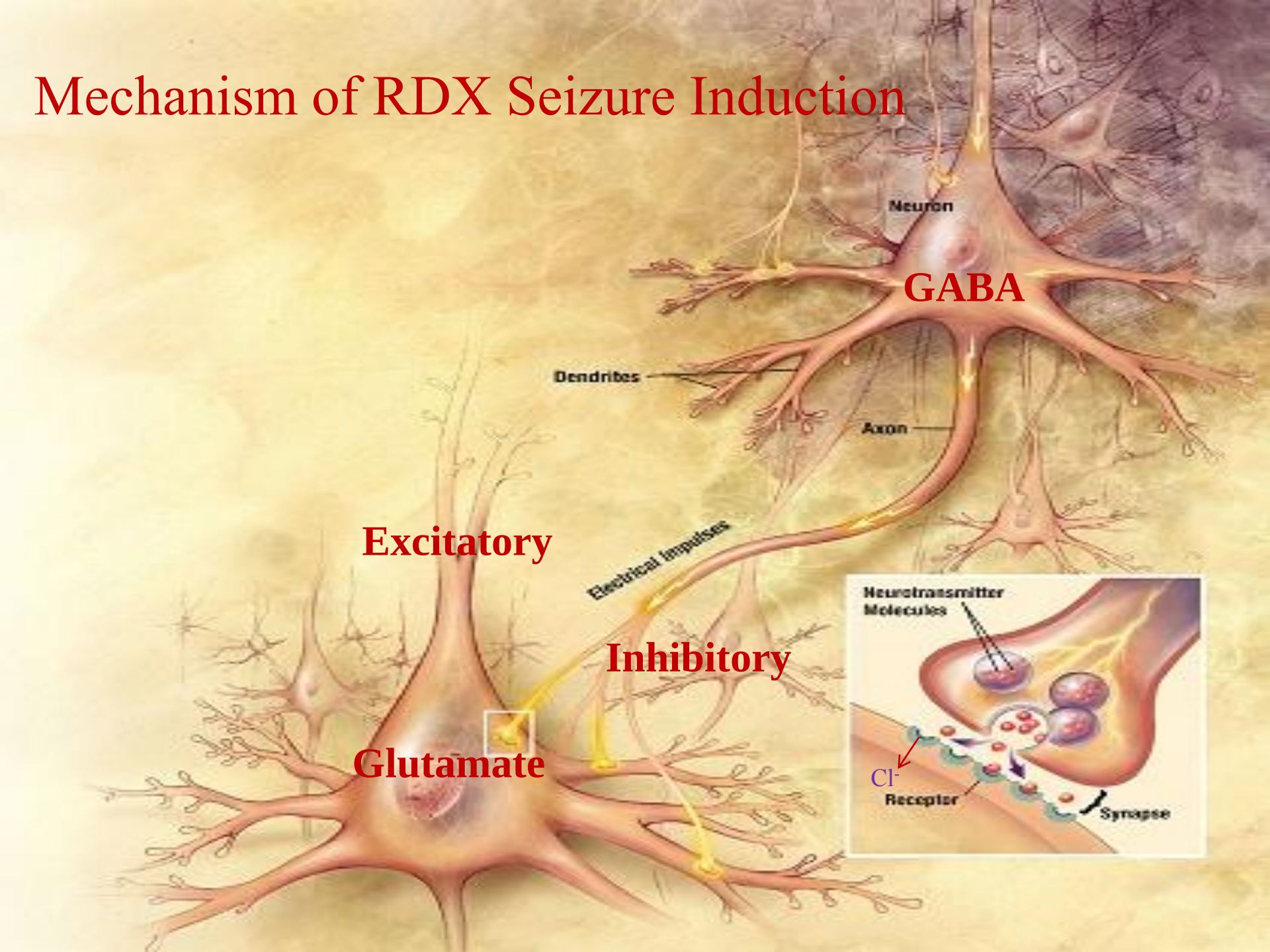
first order metabolic rate constant Kfc = 2.2 hr-1 kg-1



Interspecies extrapolation – next steps

- Determine dose to brain
- Extrapolate rat PBPK model to human
- Peer-reviewed

Mechanism of RDX Seizure Induction



GABAa Receptor

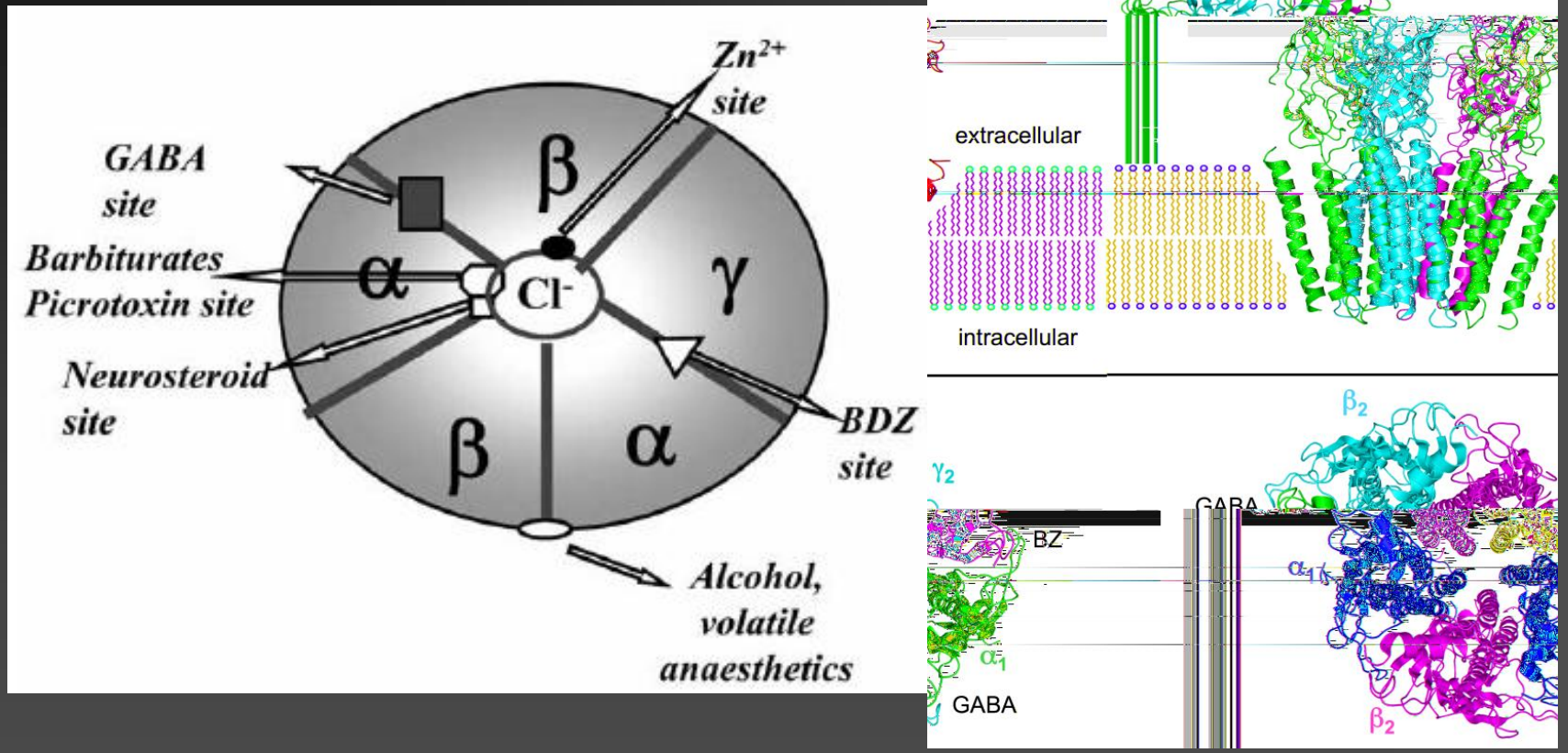
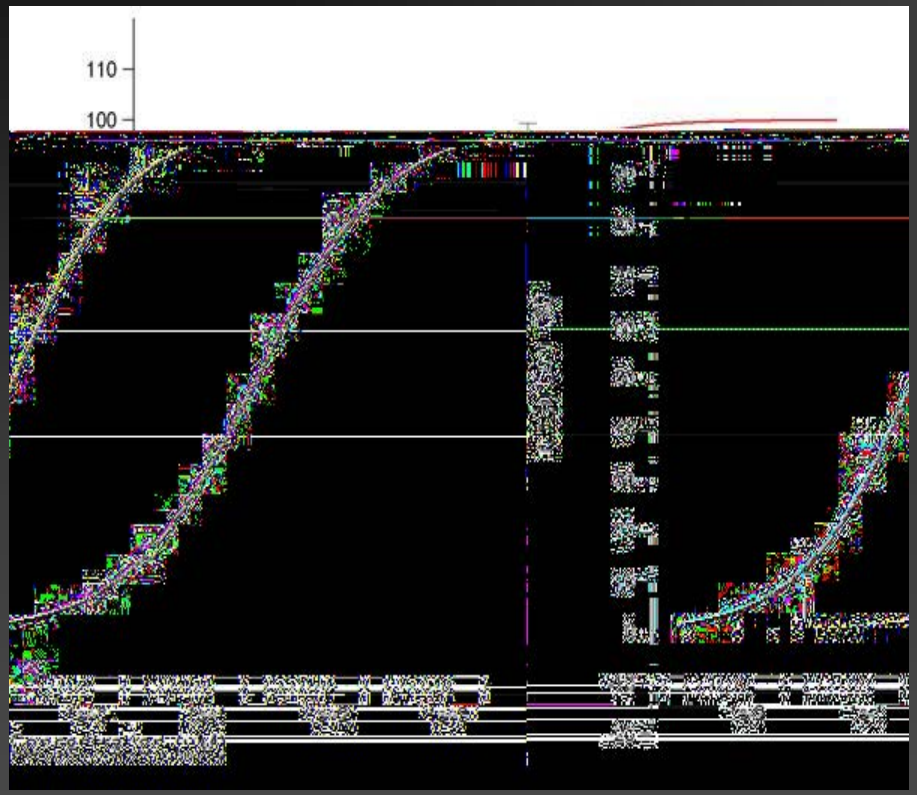


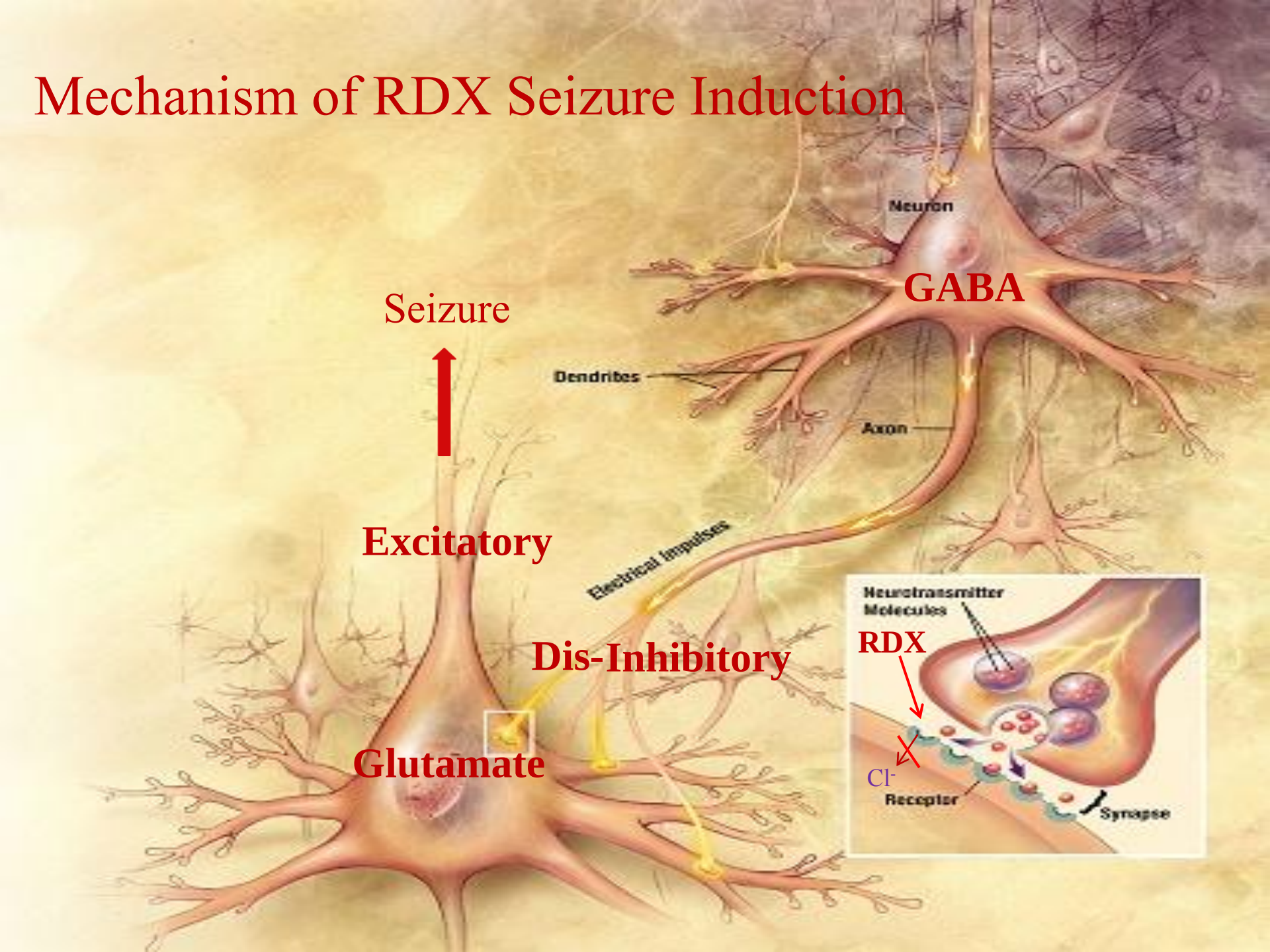
Fig. (1). Overview of the hypothetical pentameric GABAA receptor. A) Schematic representation of the GABA receptor, showing the arrangement and stoichiometry of the GABA receptor and the location of some drug binding sites. B) Representation of the GABAA receptor sitting in a cell membrane.

Effect of RDX on [^{35}S]TBPS (Convulsant Site) Binding

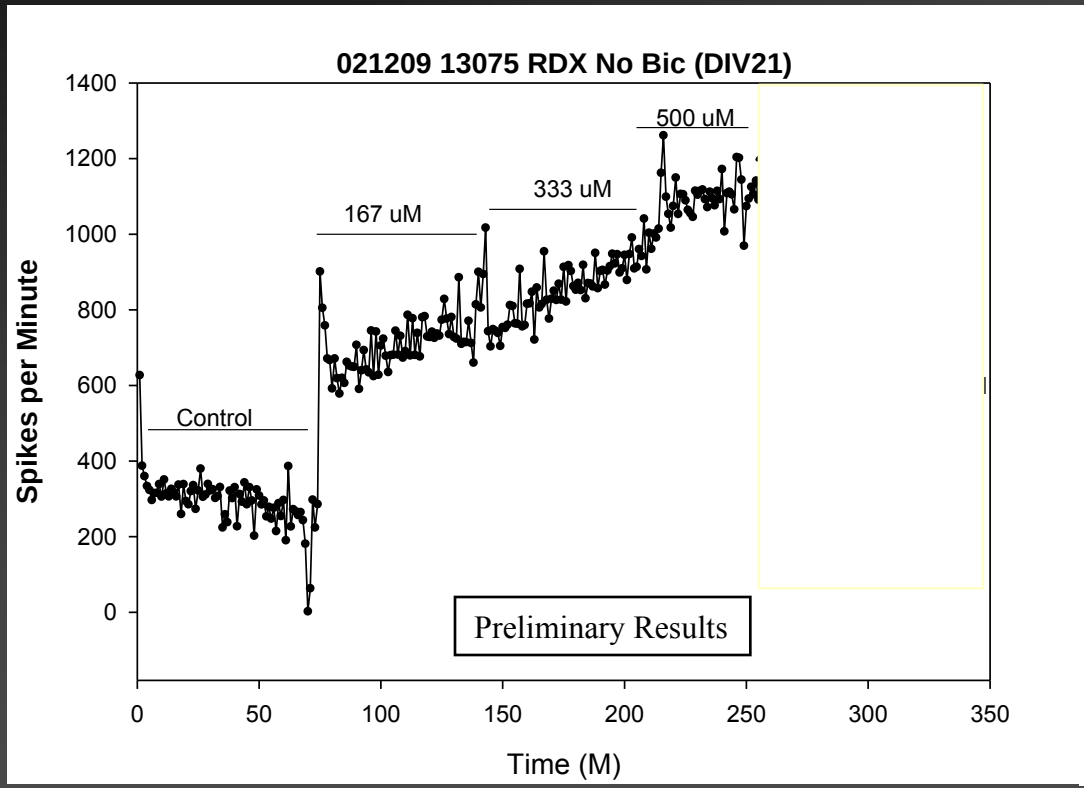


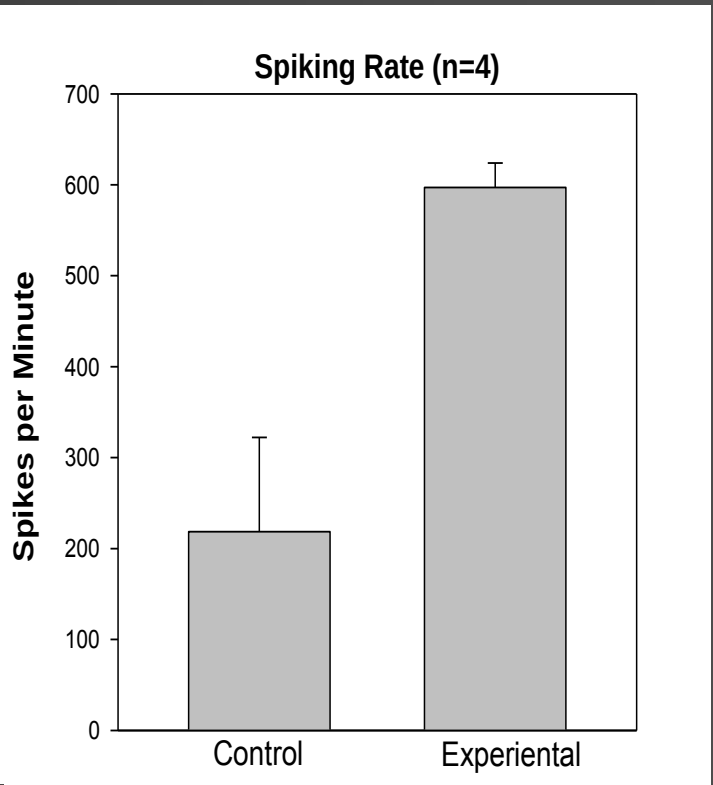
	PT	RDX
IC_{50}	0.20 µM	21.6 µM
K_i	0.20 µM	21.1µM

Mechanism of RDX Seizure Induction



Effect of RDX on Cortical Spike Activity *in vitro*





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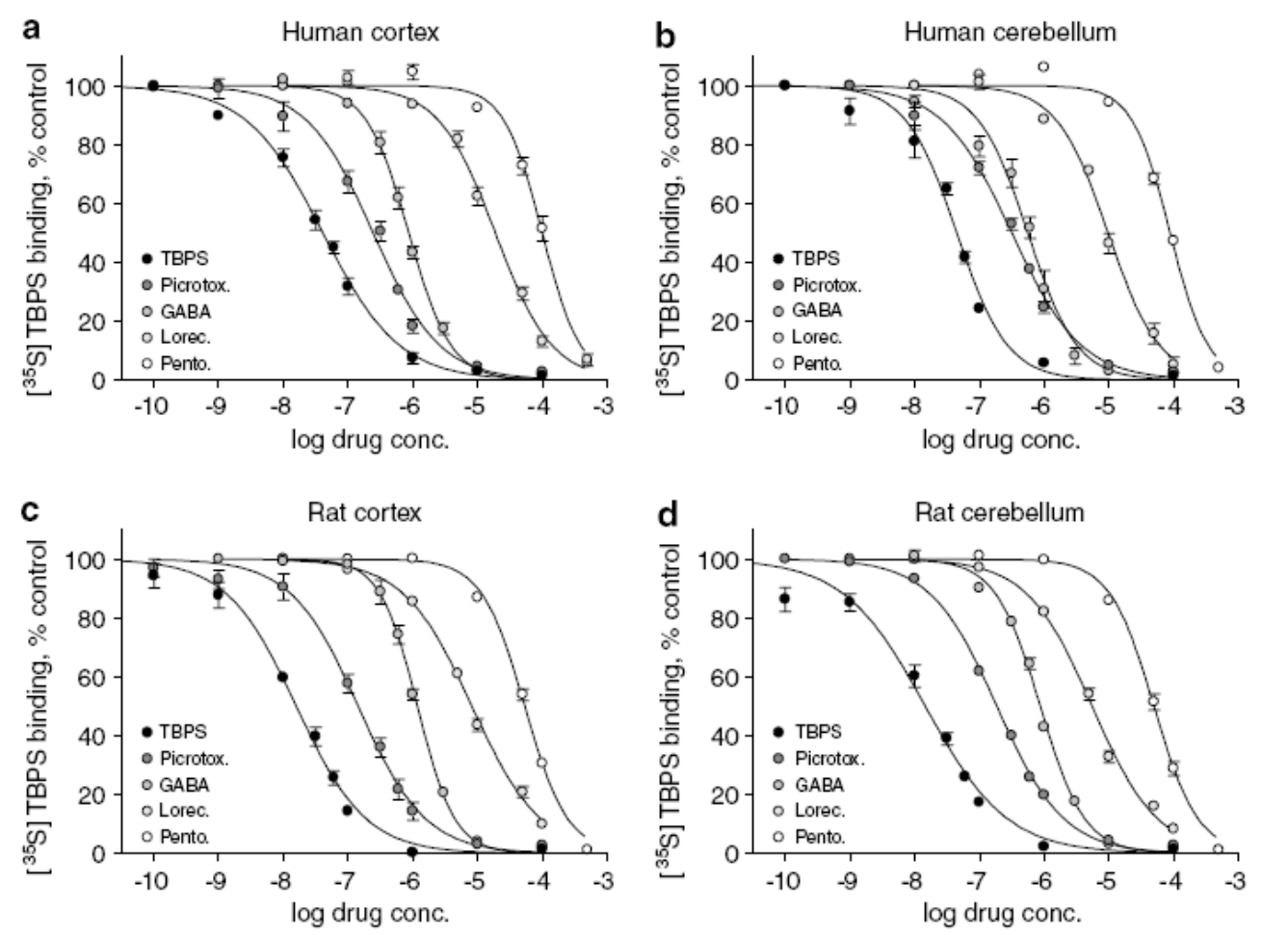
Pharmacology of Human GABAa Receptor is very similar to rat – Atack et al., 2007



If - Interspecies LOAEL/NOAEL Similar

	Western fence lizard (mg/kg-d)	Northern bobwhite (mg/kg-d)	White-footed mouse (mg/kg-d)	Fisher 344 Rat (mg/kg-d)
RDX	**5/2.5**	**8/3**	**8/4**	**8/1**

If - Interspecies GABAa Receptor Affinities Similar

Then – Interspecies UF can be reduced

Metabolism & Excretion

- Metabolism of RDX is a first order process reported to be mediated by *CYP2B4, a* phenobarbital-inducible enzyme (Bhushan et al. 2003).
- Less than 5% parent RDX eliminated in urine; majority is metabolized (Levine et al., 1977, 1978).
- Primary metabolites are NDAB & MEDINA

Completed Data

- Sub-chronic or chronic study using appropriate dose, and delivery method using at least 4 dose groups.
- Reasonable understanding of the compounds mode of action
- Metabolite study *in vivo*
- Pharmacokinetic studies and PBPK Model with 2 species validation
- Toxicodynamic assessment
- Toxicogenomic assessment
- Developmental toxicity assessment
- Reevaluation of RSC - 50% from water

Uncertainty Factors after Re-Assessment

- Animal to Human (Interspecies)
  Toxicokinetics/Toxicodynamics - 3
- Sensitive Sub-population (Intraspecies) – 10
- Level of Carcinogenicity - <10 to 0
- Relative Source Contribution – 0.5

Summary & Conclusions

- USEPA IRIS website suggests that RDX draft cancer reassessment is under agency review.
- New data that support refinement of Non-cancer Reference Dose (RfD) though the reduction of uncertainty:
  - Understanding target of toxicity.
  - Understanding absorption, distribution, metabolism, excretion and mechanism in test animals.
  - Greater confidence in understanding dose at target site and extrapolation to humans.
- Use new data to support a refined relative source contribution value for drinking water numbers.
  - Should result in raising of the Health Advisory from 2 ppb to ~5-6 ppb, μg/L.